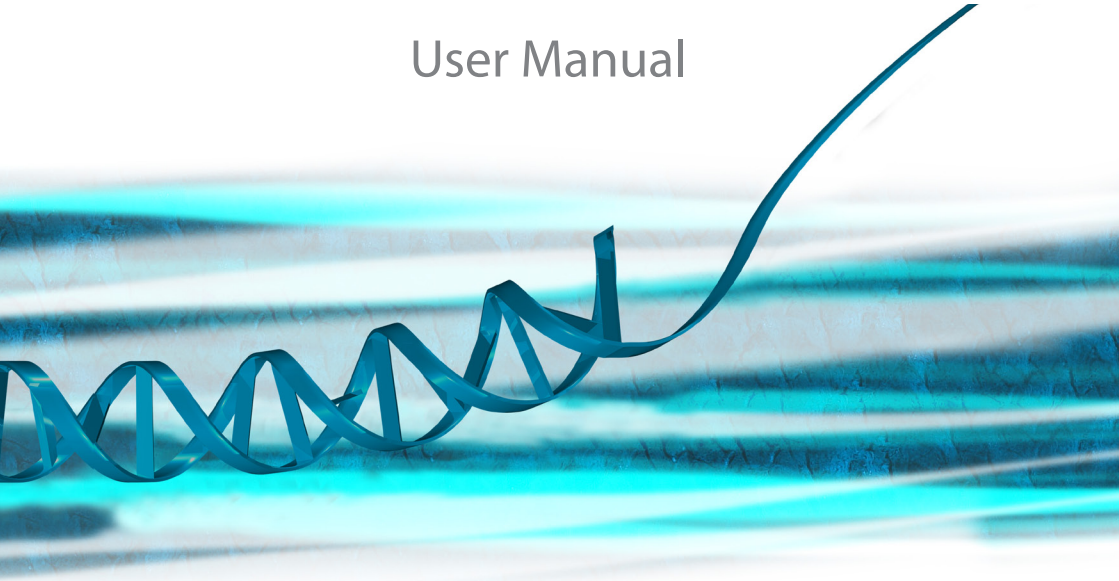
User Manual



CelluLyser™
Lysis and cDNA Synthesis Kit

Version 1.4— Oct 2012

From cells to cDNA in one tube

tataabiocenter

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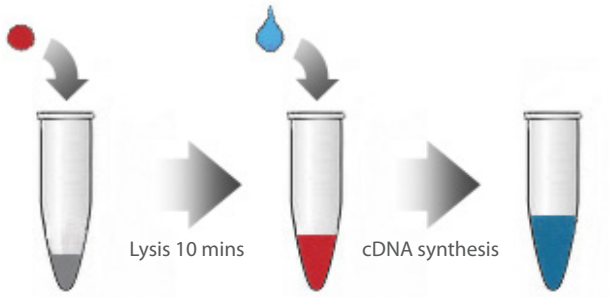
Introduction

The CelluLyser™ cDNA Synthesis Kit has been developed for fast and simple cell lysis and cDNA synthesis of small samples. With the possibility to generate cDNA directly from cell lysate without isolating RNA, considerable amount of time is saved compared to standard RNA purification. The kit comprises the CelluLyser™ buffer for a rapid and sensitive lysis of cells and cDNA synthesis reagents for reverse transcription of RNA into cDNA for samples ranging from 10 000 cells down to as little as one single cell. To avoid losses of material the entire procedure can be performed in a single tube without any sample transfer. Also, 100% of the total RNA content may be used from lysis to cDNA synthesis.

The CelluLyser™ procedure is rapid and straightforward. Cells are lysed in only 10 minutes in the optimized CelluLyser™ buffer, and thereafter sensitive cDNA synthesis is performed directly on the cell lysate.



Contents

The reagents provided are sufficient for 250 lysis reactions and 100 cDNA synthesis reactions of 1 - 2 000 cells (20 µl cDNA synthesis reactions) or 100 lysis reactions and 40 cDNA synthesis reactions of 2 000 - 10 000 cells (50 µl cDNA synthesis reactions). See A. Cell lysis on page 7 and B. cDNA synthesis on page 9 for more information.

Component	Volume	Concentration
CelluLyser™ buffer	1.25 ml	1X
cDNA synthesis reagents (Roche Transcriptor First Strand cDNA Synthesis Kit):		
-Transcriptor Reverse Transcriptase	50 µl	20 U/µl
-Transcriptor RT Reaction Buffer	1 ml	5X
-Protector RNase Inhibitor	100 µl	40 U/µl
-Deoxynucleotide Mix	200 µl	10 mM each
-Anchored-oligo(dT) Primer	200 µl	50 µM
-Random Hexamer Primer	200 µl	600 µM
-Water, PCR-grade	2x1 ml	

Storage

The CelluLyser™ buffer can be stored at 4°C for 12 months. Store the cDNA synthesis reagents at -15 to -25°C through the expiration date printed on the label. Avoid repeated freeze-thaw cycles.

Additionally required materials and devices

- General laboratory equipment including pipettes and microcentrifuge
- Nuclease-free pipette tips and microcentrifuge tubes
- Heating cabinet and ice, or thermocycler
- 4°C 1x phosphate buffered saline (PBS) (for cells requiring washing)
- For optional DNase treatment procedure: dsDNase or HL-dsDNase from ArcticZymes

Safety information

When working with chemicals, always wear a protective lab coat, disposable gloves, and protective eyewear. See the appropriate material safety sheets (MSDSs) for more information.

Procedure

Procedure overview

Lysis
1-10 000 cells are lysed in lysis buffer containing RNase inhibitors at room temperature for 10 minutes. Depending on the amount of cells or the cell vessel format, the lysis volume is 5.5 µl or 13.75 µl.

cDNA synthesis reaction is prepared
A mastermix for the cDNA synthesis is prepared appropriate for the cell lysate volume.

cDNA synthesis
The cDNA synthesis is performed either in a heating cabinet or in a thermocycler with a heated lid.

Ready cDNA
The synthesized cDNA may be stored or added to a PCR reaction without purification.

Optional DNase treatment
A separate DNase treatment to degrade contaminating genomic DNA is often not required with the CelluLyser™ Lysis and cDNA Synthesis Kit. The CelluLyser lysis buffer lyses the cells gently, releasing RNA completely from the cells without making the genomic DNA fully accessible to the DNA polymerase. If a DNase treatment of the lysate is desired, the protocol Optional DNase treatment in Appendix, page 13-17, should be followed, where the procedure is optimized for efficient removal of residual DNA.

A. Cell lysis

The protocol is optimized for use with 1-10 000 cells. Using >10 000 cells may result in incomplete lysis and/or inhibition of cDNA synthesis.

The cell lysis can be performed in either 0.2 ml PCR tubes, 384 well cell culture plates, or in 96 well cell culture plates.

Up to 2 000 cells may be lysed in 5.5 µl lysis solution. For larger number of cells (2 000-10 000) the lysis solution volume is scaled up to 13.75 µl. Additionally, if using a 96 well cell culture plate a lysis solution volume of 13.75 µl must be used to cover the well surface properly.

1. Prepare a lysis solution

Add CelluLyser™ buffer and Protector RNase inhibitor to a tube or well according to the table below. See above for guidelines about the lysis solution volume depending on cell amount and cell vessel formats.

A master mix may be prepared for multiple reactions. Use the CelluLyser™ buffer equilibrated to room temperature.

Lysis solution for 1rxn Component	1 - 2 000 cells	2 000 - 10 000 cells or 96 well cell culture plate
CelluLyser buffer	5 µl	12.5 µl
Protector RNase inhibitor	0.5 µl	1.25 µl
Total volume	5.5 µl	13.75 µl

2. Mix lysis solution and cells

Single cells (Patch clamp collection, FACS sorting, etc.)
Collect the cell with a minimal amount of residual volume and add it directly to the lysis solution. During the collection the cell should be kept in a medium or buffer that is not inhibitory to enzymatic reactions, e.g. 1X PBS buffer. Multiple cells may be added to the tube or well as long as the residual volume is low enough to avoid any significant dilution of the lysis solution which may cause incomplete lysis of the cells.

Cells in solution

a Pellet the cells and wash the pellet with 4°C 1X PBS buffer.

b Carefully remove the PBS buffer without disturbing the cells. It is critical to remove as much PBS buffer as possible, as remaining buffer will dilute the lysis solution and may cause incomplete lysis.

c Dissolve the cell pellet in lysis solution.

Adherent cells grown in 96 or 384 well cell culture plates

a Aspirate the culture media from each well. Wash the cells with 4°C 1X PBS buffer.

b Carefully remove the PBS buffer without disturbing the cells. It is critical to remove as much PBS buffer as possible, as remaining buffer will dilute the lysis solution and may cause incomplete lysis.

c Add lysis solution to each well.

3. Lysis of cells
Incubate the cells in lysis solution at room temperature for 10 minutes.

Continue with *B. cDNA synthesis* on next page, or store the lysate until the sample can be processed. The lysate may be stored at 4°C for up to two weeks or at -70°C for long term storage.

B. cDNA synthesis

cDNA synthesis is performed using the Transcriptor First Strand cDNA synthesis kit (Roche).

1. Thaw all frozen reagents before use.
Briefly centrifuge them before starting the procedure.

2. Prepare a cDNA synthesis maste rmix according to one of the tables below, depending on the cell lysate volume.

Prepare the master mix appropriate for the number of samples you are processing. Mix the reagents gently. Do not vortex.

Mastermix for 5.5 µl cell lysate Component	1rxn	Final conc.
Anchored-oligo(dT) Primer	1 µl	2.5 µM
Random Hexamer Primer	2 µl	60 µM
Water, PCR-grade	5 µl	
Transcriptor RT Reaction Buffer (5X)	4 µl	1X
Deoxynucleotide Mix	2 µl	1 mM each
Transcriptor Reverse Transcriptase	0.5 µl	0.5 U/µl
Final master mix volume	14.5 µl	

Mastermix for 13.75 µl cell lysate Component	1rxn	Final conc.
Anchored-oligo(dT) Primer	2.5 µl	2.5 µM
Random Hexamer Primer	5 µl	60 µM
Water, PCR-grade	12.5 µl	
Transcriptor RT Reaction Buffer (5X)	10 µl	1X
Deoxynucleotide Mix	5 µl	1 mM each
Transcriptor Reverse Transcriptase	1.25 µl	0.5 U/µl
Final master mix volume	36.25 µl	

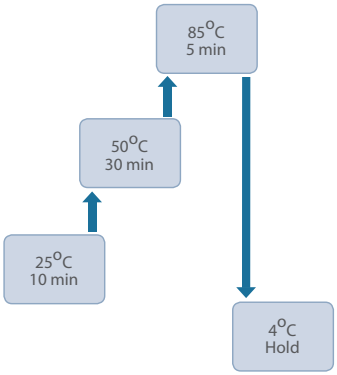
3. Add the cDNA synthesis master mix to each tube or well, either 14.5 µl to 5.5 µl cell lysate (final volume 20 µl), or 36.25 µl to 13.75 µl cell lysate (final volume 50 µl). Mix gently by pipetting. Do not vortex.

Note! A maximum of 5.5 µl lysis solution can be added to a 20 µl cDNA synthesis reaction.

4. cDNA synthesis may be performed either in a heating cabinet or in a thermocycler with a heated lid. The entire cDNA synthesis reaction can be transferred from one sample vessel to another.

If using a well plate, seal it hermetically using for example a sealing foil. This is critical to avoid evaporation during cDNA synthesis. If using a tube, make sure the lid is properly closed.

Incubate the plate or the tube according to the temperature protocol below. If using a heating cabinet, make sure that it is pre-heated before each incubation step and cool the reaction to 4°C by putting it on ice.



(If the heating cabinet cannot be heated to 85°C this step can be replaced with an incubation at 70°C for 10 minutes.)

At this point the synthesized cDNA may be stored at 2 to 8°C for several hours or at -15 to -25°C for longer periods. The cDNA can be added to a PCR reaction without purification. *Note!* Add maximum 10% of undiluted cDNA in the PCR reaction to avoid inhibiton e.g. 2 µl to a 20 µl reaction.

Troubleshooting

For problems occuring during PCR , see the instructions for the PCR kit you are using. Problems occuring during lysis and cDNA synthesis can be:

Excessive cell number (>10 000 cells)
Make sure an amount of 1-10 000 cells is used. If using >10 000 cells it may result in incomplecte lysis and/or inhibiton of cDNA synthesis.

Insufficient washing of cells
To avoid incomplete lysis, make sure cells are carefully washed with PBS and also make sure a minimal amount of residual volume is added to the lysis solution.

Insufficient volume of lysis buffer
Up to 2 000 cells may be lysed in 5.5 µl lysis solution. For larger number of cells (2 000-10 000), the lysis solution volume must be scaled up to 13.75 µl. Additionally, if using a 96 well cell culture plate, a lysis solution volume of 13.75 µl must be used to cover the well surface properly.

Insufficient volumes of cDNA synthesis reagents
Make sure the volumes of the components added to the cDNA synthesis master mix are correct. 14.5 µl master mix should be prepared for 5.5 µl cell lysate (final volume 20 µl), or 36.25 µl for 13.75 µl cell lysate (final volume 50 µl). A maximum of 5.5 µl lysis solution can be added to a 20 µl cDNA synthesis reaction.

Lysis and cDNA synthesis are not performed in referred duration times or temperatures
Make sure the CelluLyser™ buffer has been equilibrated to room temperature and that the incubation of the cells in lysis solution is performed at room temperature for 10 minutes. For the cDNA synthesis, incubate the plate or the tube according to the stated temperature protocol.

The lysate or the cDNA are not stored properly

The lysate may be stored at 4°C for up to two weeks or at -70°C for long long term storage. The synthesized cDNA may be stored at 2 to 8°C for several hours or at -15 to -25°C for longer periods.

Positive NoRT control

This is an indication of either presence of genomic DNA or contaminating DNA in the sample. Either perform the optional DNase treatment, or consider measures of DNA contamination of reagents, pipettes, benchtops etc.

Appendix: Optional DNase treatment

A separate DNase treatment to degrade contaminating genomic DNA is often not required with the CelluLyser™ Lysis and cDNA Synthesis Kit. The CelluLyser™ buffer lyses the cells gently, releasing RNA completely from the cells without making the genomic DNA fully accessible to the DNA polymerase. The level of genomic DNA contamination and the effect on gene expression analysis should be assessed for each gene using NoRT controls. A NoRT control contains all the cDNA synthesis components (including lysate) except the Reverse Transcription enzyme (substitute with water). If a NoRT control gives an amplification curve when performing downstream PCR, the signal is caused by amplification of genomic DNA. A rule of thumb is that the Cq value of the NoRT control should be at least 5 cycles higher than the Cq value of the sample that is being quantified.

If the Cq value of the NoRT control is too low, a DNase treatment of the lysate is needed. Residual DNA can be removed with the optional DNase treatment procedure, using the double strand specific DNase (dsDNase, ArcticZymes). Please note that DNase is not included in the kit.

Procedure overview

Lysis
1-10 000 cells are lysed in CelluLyser™ buffer at room temperature for 10 minutes and heated to 85°C for 5 minutes to make the genomic DNA accessible for DNase treatment. Depending on the amount of cells or the cell vessel format, the lysis volume is 5 µl or 12.5 µl. Thereafter RNase inhibitors are added.

cDNA synthesis reaction (including DNase) is prepared
A master mix for the cDNA synthesis, including the dsDNAse, is prepared appropriate for the cell lysate volume.

cDNA synthesis and DNase treatment
The cDNA synthesis and simultaneous DNase treatment are performed either in a heating cabinet or in a thermocycler with a heated lid.

Ready cDNA
The synthesized cDNA may be stored or added to a PCR reaction without purification.

A. Cell lysis

The protocol is optimized for use with 1-10 000 cells. Using >10 000 cells may result in incomplete lysis and/or inhibition of cDNA synthesis.

The cell lysis can be performed in either 0.2 ml PCR tubes, 384 well cell culture plates, or in 96 well cell culture plates.

Up to 2 000 cells may be lysed in 5 µl CelluLyser buffer. For larger number of cells (2 000-10 000) the CelluLyser buffer volume is scaled up to 12.5 µl. Additionally, if using a 96 well cell culture plate a CelluLyser buffer volume of 12.5 µl must be used to cover the well surface properly.

1. Mix CelluLyser buffer and cells

Mix pure CelluLyser buffer and cells in a tube or well according to description and add the buffer volumes stated in the table below. See *A. Cell lysis* above for guidelines about the buffer volume depending on cell amount and cell vessel formats. Use the CelluLyser buffer equilibrated to room temperature. Do not add Protector RNase inhibitors at this stage.

Lysis buffer for 1rxn Component	1 - 2 000 cells	2 000 - 10 000 cells or 96 well cell culture plate
CelluLyser buffer	5 µl	12.5 µl

Single cells (Patch clamp collection, FACS sorting, etc.) Collect the cell with a minimal amount of residual volume, and add it directly to the CelluLyser buffer. During the collection the cell should be kept in a medium or buffer that is not inhibitory to enzymatic reactions, e.g. 1X PBS buffer. Multiple cells may be added to the tube or well as long as the residual volume is low enough to avoid any significant dilution of the CelluLyser buffer which may cause incomplete lysis.

Cells in solution

a Pellet the cells and wash the pellet with 4 °C 1X PBS buffer.

b Carefully remove the PBS buffer without disturbing the cells. It is critical to remove as much PBS buffer as possible, as remaining buffer will dilute the CelluLyser buffer and may cause incomplete lysis.

c Dissolve the cell pellet in CelluLyser buffer.

Adherent cells grown in 96 or 384 well cell culture plates

a Aspirate the culture media from each well. Wash the cells with 4 °C 1X PBS buffer.

b Carefully remove the PBS buffer without disturbing the cells. It is critical to remove as much PBS buffer as possible, as remaining buffer will dilute the CelluLyser™ buffer and may cause incomplete lysis.

c Add CelluLyser™ buffer to each well.

2. Lysis of cells

Incubate cells in CelluLyser™ buffer at room temperature for 10 minutes, followed by heating to 85 °C for 5 minutes to make the genomic DNA accessible for DNase treatment. Cool the sample to 4°C.

3. Addition of RNase inhibitors

Add RNase inhibitors according to the table below. Make sure the sample has been cooled to 4°C before addition.

RNase inhibitors for 1rxn Component	5 µl CelluLyser buffer	12.5 µl CelluLyser buffer
Protector RNase inhibitor	0.5 µl	1.25 µl
Total lysate volume	5.5 µl	13.75 µl

Continue with *B. cDNA synthesis and DNase treatment* on next page, or store the lysate until the sample can be processed. The lysate may be stored at 4°C for up to two weeks or at -70°C for long term storage.

B. cDNA synthesis and DNase treatment

cDNA synthesis is performed using the Transcriptor First Strand cDNA synthesis kit (Roche). The simultaneous DNase treatment is performed using a double strand specific DNase (dsDNAse, ArcticZymes), which is specially designed for DNase treatment during the RT step. The nuclease shows 30x higher activity than bovine DNAse I and the activity towards dsDNA is 5000x higher than towards ssDNA. Please note that DNase is not included in the kit, but can be ordered from TATAA Biocenter with the kit.

1. Thaw all frozen reagents before use.
Briefly centrifuge them before starting the procedure.

2. Prepare a cDNA synthesis master mix including DNase according to one of the tables below, depending on the cell lysate volume. Prepare the master mix appropriate for the number of samples you are processing. Mix the reagents gently. Do not vortex.

Mastermix for 5.5µl cell lysate Component	1rxn	Final conc.
dsDNAse (2 U/µl)	1 µl	0.1 U/µl
Anchored-oligo(dT) Primer	1 µl	2.5 µM
Random Hexamer Primer	2 µl	60 µM
Water, PCR-grade	4 µl	
Transcriptor RT Reaction Buffer (5X)	4 µl	1X
Deoxynucleotide Mix	2 µl	1 mM each
Transcriptor Reverse Transcriptase	0.5 µl	0.5 U/µl
Final mastermix volume	14.5 µl	

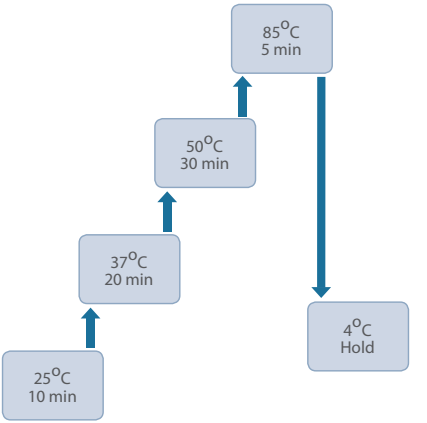
Mastermix for 13.75 µl cell lysate Component	1rxn	Final conc.
dsDNAse (2 U/µl)	2.5 µl	0.1 U/µl
Anchored-oligo(dT) Primer	2.5 µl	2.5 µM
Random Hexamer Primer	5 µl	60 µM
Water, PCR-grade	10 µl	
Transcriptor RT Reaction Buffer (5X)	10 µl	1X
Deoxynucleotide Mix	5 µl	1 mM each
Transcriptor Reverse Transcriptase	1.25 µl	0.5 U/µl
Final mastermix volume	36.25 µl	

3. Add the cDNA synthesis master mix to each tube or well, either 14.5 µl to 5.5 µl cell lysate (final volume 20 µl), or 36.25 µl to 13.75 µl cell lysate (final volume 50 µl). Mix gently by pipetting. Do not vortex. *Note!* A maximum of 5.5 µl lysis solution can be added to a 20 µl cDNA synthesis reaction.

4. cDNA synthesis may be performed either in a heating cabinet or in a thermocycler with a heated lid. The entire cDNA synthesis reaction can be transferred from one sample vessel to another.

If using a well plate, seal it hermetically using for example a sealing foil. This is critical to avoid evaporation during cDNA synthesis. If using a tube, make sure the lid is properly closed.

Incubate the plate or the tube according to the temperature protocol below. If using a heating cabinet, make sure that it is pre-heated before each incubation step and cool the reaction to 4°C by putting it on ice.



(If the heating cabinet cannot be heated to 85°C this step can be replaced with an incubation at 70°C for 10 minutes.)

At this point the synthesized cDNA may be stored at 2 to 8°C for several hours or at -15 to -25°C for longer periods. The cDNA can be added to a PCR reaction without purification. *Note!* Add maximum 10 % undiluted DNA to the PCR reaction i.e. 2 µl to a 20 µl reaction to avoid inhibition.

Contact and ordering information

To reorder or for more information about the product and other products available from TATAA Biocenter, please contact us on order@tataa.com or visit our website www.tataa.com.

CelluLyser Lysis and cDNA Synthesis Kit	CelluLyser buffer (250 or 100 rxns*) and cDNA synthesis reagents (100 or 40 rxns*) *the amount of reactions are depending on cell amount and vessel format	Order# H101

License information

The purchase of this product conveys to the buyer the non-transferable right to use the purchased amount of the product and components of the product in research conducted by the buyer (whether the buyer is an academic or for-profit entity). The buyer cannot sell or otherwise transfer (a) this product (b) its components or (c) materials made using this product or its components to a third party or otherwise use this product or its components or materials made using this product or its components for commercial purposes. For information on purchasing a license to this product for purposes other than research, contact TATAA Biocenter AB, Odinsgatan 28, S-41103 Göteborg, Sweden, Phone: +46 31 761 57 00, Fax: +46 31 152890, Email: info@tataa.com

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Other products from TATAA

dsDNase

DNase enzyme from ArcticZymes which is specific to double stranded DNA. Double strand specific nuclease is efficiently inactivated at 65°C and can be included in your RT reaction without degrading the single-stranded cDNA

(HL-)dsDNase

New generation DNase enzyme from ArcticZymes which is specific to double stranded DNA. Heat Labile-double strand specific nuclease is efficiently inactivated at 55°C and can be included in your RT reaction without degrading the single-stranded cDNA.

Reference Gene Panel - Human or Mouse

The panel contains primer sets for 12 commonly used human or mouse reference genes. A perfect product for fast detection and confirmation the most optimal reference genes for your samples. A one year licence for GenEx Standard software with GeNorm and Normfinder is included in the kit.

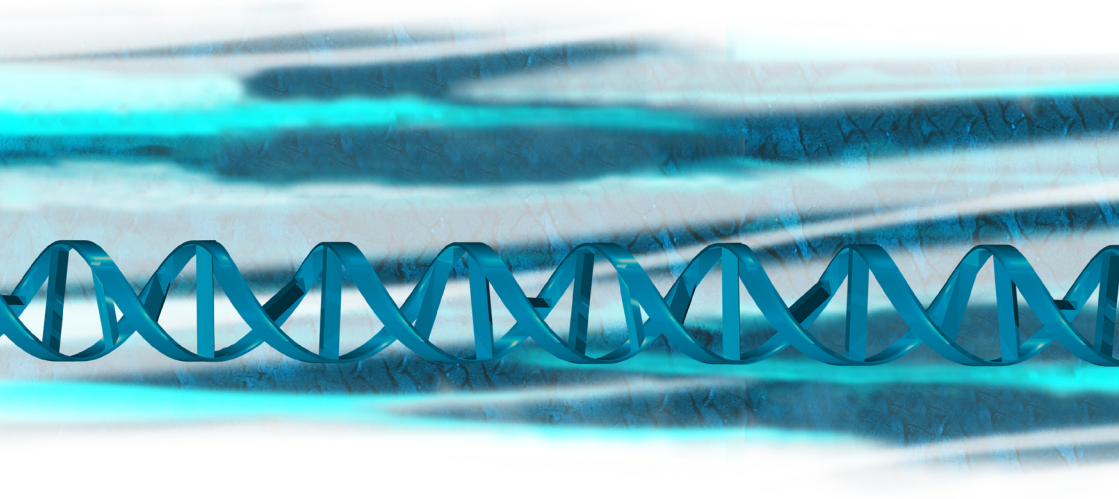
GenEx software

A software developed for gene expression analysis. GenEx provides the appropriate tools to analyze real-time PCR gene expression data and to extract valuable information from the measurements.

VisiBlue™ qPCR mix colorant

The VisiBlue qPCR mix colorant enables you to quickly color your favourite qPCR mastermix to easily visualize where the reagent has been added to your plates and tubes. VisiBlue is easy to use by a simple addition to your master mix.

Express your genius



TATAA Biocenter, with offices in Gothenburg, San Francisco and Prague is the leading provider of real-time PCR services and the prime organizer of real-time PCR workshops globally. TATAA Biocenter conducts commissioned research and training within the field of molecular diagnostics and gene expression analysis, along with developing real-time PCR expression panels. TATAA Biocenter has great experience and expertise in high resolution gene expression profiling, pathogen detection, and small sample/single cell analysis.

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